

Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma* infection in the domestic pig

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Abstract

The modified agglutination test (MAT) and a commercially available enzyme-linked immunosorbent assay (ELISA) were compared for detection of antibodies to *Toxoplasma gondii* in naturally-infected market-aged pigs. Infected pigs were obtained from commercial slaughter facilities and from farms where infection had previously been detected. Infection was confirmed by bioassay in cats. For 70 bioassay positive pigs, 60 were positive by MAT (85.7% sensitivity) and 62 were positive by ELISA (88.6% sensitivity). Of 204 bioassay negative samples 193 were negative by MAT (94.6% specificity) and 200 were negative by ELISA (98.0% specificity). Good correlation was seen between MAT and ELISA results. The results suggest that the ELISA may be a good tool for epidemiological studies of *Toxoplasma* infection on pig farms.

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1. Introduction

Toxoplasma gondii is responsible for approximately 20% of all deaths attributed to foodborne pathogens in the US, and the Centers for Disease

Control estimate that 50% of all human exposures to *T. gondii* are foodborne (Mead et al., 1999). This estimate is based on various sources of information. For example, in a cross-sectional study of adults, a group known to avoid eating meat (Seventh Day Adventists) had a significantly lower prevalence rate of infection (18%) as compared with the non-Seventh Day Adventists in the study (40%) (Rogghmann et al., 1999). Of the major meat animal species investigated

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thus far, pigs are the only species shown to frequently harbor *T. gondii* (Dubey, 1986) and therefore, pork may pose a risk to humans for exposure to *T. gondii*.

Prevalence of *T. gondii* in pigs varies, but generally exceeds 10–20% in most countries. Infection rates are higher in breeding populations than in market-aged pigs, indicating that length of exposure is a factor in acquiring *T. gondii* infection. In the US, infection was estimated at 23.9% of pigs in 1983–1984 with higher rates in breeders (42%) than in market pigs (23%) (Dubey et al., 1991). When pigs from these same areas were tested in 1992, the infection rate had dropped to 20.8% of breeders and 3.1% of finisher pigs (Dubey et al., 1995). Prevalence of *T. gondii* was 20% in sows tested in the 1990 National Animal Health Monitoring System (NAHMS) swine survey (Patton et al., 1996). Using sera from the NAHMS swine survey conducted in 1995, sow prevalence had fallen to 15.0% and finisher pigs had a seroprevalence of 3.2% (Patton et al., 2000).

There are no programs for the slaughter inspection of pigs for *T. gondii* because it is not possible to detect the microscopic tissue cysts by visual inspection. Methods for testing pigs, including serology and bioassay, are either not suitable or not sufficiently reliable for purposes of meat inspection. However, serology testing is a useful method for epidemiological purposes and for estimating infection rates by farm, herd or region. Assessment of infection rates, coupled with efforts to reduce infection by improved management on the farm, is the best way to reduce potential exposure of consumers to *T. gondii* in pork.

Serological assays include various forms of agglutination tests and the enzyme-linked immunosorbent assay (ELISA). One test that has been reported to be both sensitive and specific is the modified agglutination test (MAT) using preserved whole tachyzoites (Desmonts and Remington, 1980; Dubey et al., 1995). This test, however, is not suitable for use

in the slaughterhouse or in the field due to the length of time required to obtain a result. The availability of an ELISA that is both sensitive and specific would allow wider use of serologic testing. A few studies have compared ELISA tests with the MAT for detection of *T. gondii* infection in pigs (summarized in Table 1). These comparisons have generally used bioassay, in which portions of tissue are inoculated into mice or cats, as the gold standard. While the ELISA looks promising from these studies, further testing of naturally infected pigs is needed. In the present study, we compare a commercially available ELISA with the MAT using sera from pigs with naturally acquired *T. gondii* infections.

2. Materials and methods

Serum, meat juice, and tissue samples (heart and tongue) were obtained from random carcasses in a commercial slaughter facility and from market-aged (5–7 month old) pigs from selected herds in the northeastern United States. For random slaughter samples, 300 hearts and diaphragm tissue samples were collected each week for 13 weeks (April–August, 1999) from a large pork processing plant in the northeastern US. Serum samples were prepared from heart blood as described by Dubey et al. (1995). Meat juice samples were prepared from diaphragm tissue as described by Gamble and Patrasco (1996). Presumptive testing of these 3900 samples was performed using the MAT (Dubey et al., 1995). Samples from all MAT-positive pigs and samples from three times this number of MAT-negative samples were retained for further testing.

Additional samples from *T. gondii*-positive pigs were collected during November 2001 through January 2002 on pork production sites in the New England states where infection was known to occur

Table 1

Previous studies comparing the sensitivity and specificity of MAT and ELISA for detection of *Toxoplasma gondii* infection in pigs

Reference	MAT		ELISA	
	Sensitivity	Specificity	Sensitivity	Specificity
Waltman et al. (1984)	ND	ND	88.2–97.4	77.1–100
Dubey et al. (1995)	82.9	90.3	72.9	85.9
Wingstrand et al. (1997)	ND	ND	95.1	ND
Lind et al. (1997)	ND	ND	94.0	92.0

Table 2

MAT titers and ELISA results for 70 bioassay-positive pigs and 204 bioassay-negative pigs

Samples	MAT (titer)					ELISA	
	0	1:10	1:25	1:100	1:500	Positive	Negative
Bioassay positive ($n = 70$)	3	7	35	20	5	62	8
Bioassay negative ($n = 204$)	175	18	10	1	0	4	200

from prior epidemiological studies (Dubey et al., 2003; Gamble et al., 1999; Lehmann et al., 2003). Blood was collected from ear-tagged pigs, on-farm, by jugular venipuncture; the serum was separated, and presumptive testing was performed by MAT, as above. MAT-positive farm pigs, and a group of three times this number of MAT-negative farm pigs, were slaughtered at commercial facilities and tissues collected and processed as described above.

Heart tissue from MAT-positive pigs and MAT-negative pigs was tested by bioassay in cats (Dubey et al., 1995). For MAT-positive pigs, 100 g of heart tissue was fed individually to parasite-free cats. Feces of these cats were examined for *T. gondii* oocysts (Dubey et al., 1995) from 3–10 days post-feeding. Heart tissue from MAT-negative pigs was pooled (five hearts/pool) and fed to cats (500 g total) and feces examined for oocysts from 3–10 days post-feeding. When cats fed tissue from MAT-negative pools shed oocysts, heart samples from the pool were re-fed to cats individually.

Serum and tissue fluid samples from all bioassay positive and bioassay negative pigs were tested by MAT and ELISA. The MAT was performed as described above, at serum dilutions of 1:10, 1:25, 1:100 and 1:500. A titer of 1:25 was considered positive and a titer of 1:10 was considered suspect. Serum and tissue fluid samples were tested by ELISA using a commercial test kit (*Toxoplasma* Microwell Immunoassay Kit, Safe-Path Laboratories, Carlsbad, CA). This kit uses formalin-fixed whole tachyzoites as antigen. Serum samples were diluted 1:100 and tissue fluid samples were diluted 1:10. Kits were modified for additional testing by changing the antigen to a commercially-produced P30 antigen (Advanced Immunochemical, Long Beach, CA). The kits were optimized for this antigen using a standard panel of positive and negative samples.

Based on estimates of sensitivity and specificity obtained with the MAT and ELISA, positive and

negative predictive values were calculated using the most recently reported national estimate for prevalence of *T. gondii* in market hogs.

3. Results

A total of 274 samples were selected for testing by bioassay, MAT and ELISA. The results of these tests are shown in Tables 2 and 3, and Fig. 1.

Of 70 serum samples from pigs that were positive for *T. gondii* by bioassay, 60 were positive by MAT using a 1:25 titer as the positive cut-off (85.7%

Table 3

Positive (PPV) and negative predictive values (NPV) for the MAT and ELISA based on specificity and sensitivity in this study

Samples		MAT	ELISA
High prevalence (47.4%)	PPV	93.5	97.6
	NPV	88.0	90.5
Low prevalence (0.14%)	PPV	2.1	5.7
	NPV	99.9	99.9

Values are calculated for a high prevalence population –47.4% infection rate as found in the northeast US (Gamble et al., 1999) and a low prevalence population –0.14% infection rate as found in mid-West confinement systems (Gamble et al., unpublished).

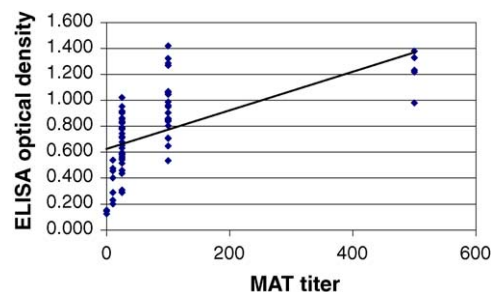


Fig. 1. Relationship between MAT titer and ELISA optical density for 70 bioassay positive pigs. Data shown for individual pigs was fitted to a linear regression.

sensitivity). If samples having an MAT titer of 1:10 are included, 67 samples were positive, increasing the sensitivity to 95.7%. A total of 62 samples tested positive by ELISA using serum, for a sensitivity of 88.6%. The eight samples testing negative had optical density (O.D.) values ranging from 0.124–0.292. These samples had corresponding MAT's of 0 (three samples), 1:10 (three samples) and 1:25 (two samples). The relationship of MAT titers to ELISA O.D. values is shown in Fig. 1. A positive correlation was seen between MAT titer and ELISA O.D.

Of 204 samples testing negative in cat bioassay, 193 were negative by MAT using a positive cut-off of a 1:25 titer. This gave a specificity of 94.6%. Of the samples testing negative, 175 had titers of 0 and 18 had titers of 1:10. Only 4 of 204 bioassay negative samples were positive by ELISA (specificity of 98.0%). The four ELISA positive samples had O.D. values of 0.476, 0.504, 0.460 and 0.321; these samples all had corresponding MAT titers of 1:25.

The ELISA test using tissue fluids from bioassay positive pigs was not as effective in detecting antibodies to *T. gondii* as testing of serum samples. Only 42 of 70 samples yielded O.D. values above the ELISA positive cut-off for a test sensitivity of 60.0%. Only 40 of 70 *T. gondii* bioassay positive samples were detected by ELISA when the crude tachyzoite antigen was replaced with a commercial P30 antigen (sensitivity = 57.1%).

Positive and negative predictive values for the MAT and ELISA were calculated for high risk and low risk populations using the sensitivity and specificity obtained in this study (Table 3). Consistent with higher sensitivity and specificity, the PPV and NPV for the ELISA are better than the MAT. However, in populations where the true prevalence is very low, such as pigs raised in confinement management systems, the positive predictive value is poor for either MAT or ELISA.

4. Discussion

In this study, we compared the MAT and the ELISA for the ability to detect antibodies to *T. gondii* in naturally-infected market-aged pigs. The ELISA, using a whole-tachyzoite antigen, performed slightly better than the MAT in detecting antibodies to *T.*

gondii in naturally infected pigs (88.6% sensitivity for the ELISA and 85.7% sensitivity for the MAT). Some false-positive results were obtained with both tests, but the specificity of the ELISA was better (98.0%) as compared with the MAT (94.6%). Because the cat bioassay is considered the “gold standard”, all measures of sensitivity and specificity are dependent upon the accuracy of this method. Of the four ELISA positive/bioassay negative samples, all had MAT titers of 1:25. These results suggest that either (1) specific antibodies were present in pig sera from these four animals, but the infection level was sufficiently low that a 100 g tissue sample did not contain tissue cysts, or (2) both tests recognized cross-reactive antibodies. With bioassay positive sera, good correlation was obtained between MAT titer and ELISA optical density.

Most previous studies using ELISA to detect *T. gondii*-specific antibodies have examined antibody responses in experimentally infected-pigs (Waltman et al., 1984; Lind et al., 1997; Wingstrand et al., 1997). Under these controlled conditions, relatively good results have been obtained in terms of sensitivity and specificity. One previous study (Dubey et al., 1995), using naturally infected sows, found the MAT to be superior to an ELISA. Results from that study were based in part on bioassays conducted in mice, a method which has lower sensitivity when compared with bioassay in cats. Further, sera collected from sows were hemolyzed, contained microbial contamination in some cases, and was subjected to repeated freeze-thaw cycles, all factors which contribute to poor performance in the ELISA. In the present study, we used fresh sera in a commercial ELISA which had been optimized for consistent performance.

In previous studies (Gamble, unpublished) we tested tissue fluids with the same ELISA used in this study and had some success in identifying infected herds. The results presented here, suggest that the sensitivity of the *T. gondii* ELISA using tissue fluids is considerably less than obtained when testing serum samples. Therefore, estimates of within herd prevalence based on ELISA testing using tissue fluids are likely to be lower than the true prevalence. The lower sensitivity of the *Toxoplasma* ELISA using tissue fluids contrasts with comparable sensitivity of testing using swine sera and tissue fluids in an ELISA for

Trichinella antibodies in pigs (Gamble and Patrascu, 1996).

In another study, recombinant P30 antigen was effective in detecting antibodies to *T. gondii* in experimentally infected pigs (Gamble et al., 2000). Initial testing with P30 antigen in this study resulted in good recognition of antibodies in a panel of positive sera (data not shown). However, when used to test sera from naturally-infected pigs, the sensitivity of the test was reduced to an unacceptable level.

Based on the results of this study, the ELISA test performs as well as, or better than, the MAT for detecting serum antibodies to *Toxoplasma* in pigs. Considering the length of time needed to perform the MAT and the difficulty in interpreting the results, the ELISA appears to be a more useful test for routine screening of pigs on the farm or at slaughter facilities. However, due to a low rate of false-negative results, neither test would be suitable for individual carcass testing for purposes of assuring food safety. Further, in populations where the prevalence is extremely low neither test is a good predictor of true positives.

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